SOX10

Gene Function

Analysis of a variety of *SOX10* mutation constructs, including those occurring in human WS4 individuals, allowed functional information about SOX10 protein to be revealed. The data showed that the **HMG domain is required for nuclear localization and DNA binding, the amino terminal 60 residues function for synergistic transcription activation with *Pou3f1/SCIP*, and the C-terminus functions as a transactivation domain.** Modest transcriptional activity of SOX10 in transient transfection luciferase assays is reported relative to other transcription factors, suggesting that achievement of higher levels by endogenous SOX10 requires combination with other transcription factors (Kuhlbrodt et al., 1998a, Kuhlbrodt et al., 1998b).

SOX proteins typically require other proteins to bind DNA as cofactors. SOX10 and other SOXE proteins (SOX 8, 9, and 10) are monomeric in solution, and presumably bind DNA as a monomer. Interestingly, **several target promoters of SOX10 contain dimeric binding sites** that allow SOX10 molecules to undergo cooperative binding and functional dimerization on DNA (Peirano and Wegner, 2000, Schlierf et al., 2002). The **monomeric and dimeric binding sites do not appear to be functionally interchangeable**, suggesting that SOX10 may regulate target genes through two distinct modes of action that are dependent on monomeric versus dimeric binding (Schlierf et al., 2002).

Binding of SOX proteins can confer conformational changes on DNA. Structural studies show that SOX proteins make key contacts with the minor groove of DNA, and subsequently widen the minor groove to cause a 70-85° bend in the DNA (Connor et al., 1994, Werner et al., 1995). This suggests SOX10 acts as an architectural protein, which is of particular interest where multiple SOX10 binding sites are located together (i.e. in *Mitf* and *Dct* promoters), potentially causing distinctive changes in the 3-dimensional structure of the promoter or enhancer and all associated binding factors (Wegner, 2005).

SOXE proteins **contain two nuclear localization signals as well as a nuclear export signal**, suggesting that they shuttle back and forth between the nucleus and the cytoplasm, facilitating sequestration for tight regulation of SOX proteins (Wegner, 2005).

Mutation of *Sox10* disrupts neural crest-derived melanocyte development. The spontaneous mutant *Dominant megacolon (Dom)*, which exhibits white spotting and megacolon with dominant inheritance in heterozygotes and embryonic lethality in homozygotes, is caused by mutation of *Sox10* (Southard-Smith et al., 1998).

Analysis of melanocyte development in ***Sox10^{LacZ}*** **transgenic mice demonstrated that loss of *Sox10* results in severe loss of melanoblasts**, as measured by absence of *Sox10^{LacZ}*-, *Mitf*-, or *Dct*-positive cells in *Sox10^{LacZ}/Sox10^{LacZ}* homozygotes. Cells expressing *c-Kit* were also reduced, to 24% of wild type in homozygotes and 50% in heterozygotes. Heterozygous *Sox10^{LacZ}*/+ mice also showed 50% reduction in *Mitf*-positive cells; however *Dct*-expressing cells were reduced to 12% of wild type in heterozygotes (Britsch et al., 2001).

Studies of retrovirally-infected, cultured mouse neural crest stem cells suggest that SOX10 has multiple functions in maintaining stem cell properties in developing neural crest cells. **SOX10 maintains the multipotent state of neural crest cells**, preserving their ability to differentiate into neurons or glia, as measured by the ability of SOX10 expression to prevent the extinction of these lineage potentials by expression of factors that commit cells to a particular fate. **Also, SOX10 inhibits differentiation into neuronal or smooth muscle lineages**, however this function is dosage dependent, requiring higher SOX10 levels than those for maintaining neural crest multipotency. SOX10 expression also prevents TGF-b-induced cell cycle arrest (Kim et al., 2003).

Generation of two *Sox10* hypomorphic mutant alleles revealed new functional information about *Sox10*. **The *Sox10* aa1 mutant, which introduces 3 alanine residues within the conserved dimerization domain, blocked formation of DNA-dependent SOX10 dimers; the *Sox10* DK2 mutant, which deletes a conserved region 3 of the HMG domain (amino acids 233-306), showed selective reduction in transactivation activity on the *Dct* (and *Mpz*) promoters**. Some aa1 heterozygotes displayed a white belly spot, and aa1 homozygotes showed a nearly complete absence of *Dct*-positive melanoblasts at E12.5, indicating the melanocyte lineage is absent. The DK2 heterozygotes never showed a white belly spot, and homozygous DK2 mutants showed an 80% reduction in *Dct*-positive melanoblasts at E12.5. **These data suggest that both the dimerization and the K2 domains of SOX10 are functional in regulating melanocyte development; however the dimerization domain is of greater importance**. Enteric nervous system development along with later developmental stages of satellite glia and Schwann cells were also affected in both *Sox10* mutants (Schreiner et al., 2007).

SOX10 regulates inner ear development by promoting the survival of cochlear progenitor cells during development of the otocyst; this function may be mediated through SOX10 activation of *Jagged1*, a gene that activates the Notch signaling pathway and is necessary for development of the inner ear prosensory domain, as SOX10 activated the *Jagged1* promoter in luciferase assays (Breuskin et al., 2009).

In ovo electroporation of a variety of human *SOX10* constructs into the neural crest of chick embryos was used to identify the specific protein regions of SOX10 required at various stages of neural crest development. The DNA-binding domain and the transactivation domain were shown to be essential for neural crest induction, while the nuclear export signal and the N-terminal region (amino acids 1-60) were not essential for neural crest induction. The SOXE conserved dimerization domain (amino acids 61-100) was required for early neural crest induction, but to a lesser extent needed for gene induction; similarly the SOXE conserved transactivation domain (amino acids 234-306) was required to a lesser extent for both neural crest induction and gene expression. Extensive analysis of specific mutations found in human WS patients and the *Sox10^{Dom}* mouse model elucidated mechanistic details of the various SOX10 proteins. These details included description of an absence of function in a majority of mutations, and a predicted limitation of dominant-negative effects to specific lineages during later developmental stages (Cossais et al., 2010).

Culturing human embryonic stem cells (hESC) in the absence of feeder cells demonstrated that **hESC neurospheres are SOX10-negative,** but PAX3- and SOX9-positive (approximately 80% and 55% of cells, respectively). **When hESC neurospheres are plated onto fibronectin, early emigrating neural crest stem cells uniformly begin to express SOX10 protein.** Gene expression characteristics and differentiation potentials of these SOX10-positive emigrating neural crest stem cells were examined in detail (Curchoe et al., 2010).